EUS-FNA OF PANCREATIC EXOCRINE TUMORS
COMPARISON OF EXPERIENCES WITH PATHOLOGICAL DIAGNOSIS

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Goals of Talk

- Brief overview: pathology of exocrine pancreatic tumors

- The viewpoint of the pathologist: mini-review of literature on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of EUS-FNA Cytology of exocrine pancreatic tumors

- Presentation of a case of rare exocrine tumor, diagnosed by EUS-FNAC with immunocytochemistry

- Relevance of new methods of diagnosis
Background - 1

- **EUS-FNAC** is currently being applied for the preoperative diagnosis of pancreatic exocrine tumors with limited pitfalls.

**BENIGN OR BORDERLINE TUMORS**

- **Cytological diagnosis is reliable.** The most problematic discernment is between serous and mucinous cysts, with the latter considered premalignant. Pseudocysts may also be difficult to diagnose. Cystic fluid may be analyzed for cytology, biochemistry and tumor markers.

Song et al, Gastr Endosc 2003  
Chang, Endoscopy 2006  
Rocca et al, Dig and Liv Dis 2007
In the past few decades there have been relatively few changes in the epidemiology, therapy and overall survival of pancreatico-biliary tumors.

Current diagnostic and therapeutic approaches have the potential of favorably impacting on the natural history of these tumors.

It is essential that diagnostic tests are accurate with a high negative predictive value to avoid missing potential resectable tumors.

Chang, Endoscopy 2006
**FNAC during EUS** is one of them, having a relevant role in the diagnosis and staging of pancreatic cancer even when prior biopsy techniques have been unsuccessful.

Preoperative diagnosis of malignancy enables the surgeon to plan the operative procedures, whether it be resection or diversion, and it can help avoid surgery in patients unlikely to tolerate a surgical procedure.

In protocols utilizing preoperative neoadjuvant therapy in potentially resectable patients, the cytological diagnosis of pancreatic malignancy is essential.

Suits et al Arch Surg 1999
Harewood et al, Am J Gastroenterol 2002
Chang, Endoscopy 2006
<table>
<thead>
<tr>
<th>Cystic</th>
<th>and</th>
<th>Solid</th>
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<tbody>
<tr>
<td>Serous Cystadenoma</td>
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<tr>
<td>Mucinous Cystic Neoplasm</td>
<td>□ Mucinous Cystadenoma</td>
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<tr>
<td></td>
<td>□ Borderline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>□ Cystadenocarcinoma</td>
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<tr>
<td>Intraductal Papillary Mucinous Neoplasm</td>
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**Solid Pseudo papillary Tumor**

- Ductal adenocarcinoma
  - Undifferentiated carcinoma with or without osteoclastic-like giant cells
  - Adenosquamous carcinoma
  - Signet ring carcinoma
  - Mucinous non-cystic carcinoma
  - Spindle cell carcinoma
  - Mixed ductal-endocrine ca.

- Acinar cell carcinoma
- Medullary carcinoma
- Clear cell carcinoma
- Ciliated cell carcinoma

PanIN –IA, PanIN-IB, PanIN-II, PanIN- III: carcinoma in situ
Serous Cystadenoma

25% of all cystic pancreatic tumors
Benign; 11 cases described with malignant behavior*

Cytological findings

- Usually hypocellular
- Epithelial cells are cuboidal or low columnar with bland grooved nuclei
- Cellular arrangement may include flat sheets with a honeycomb pattern, small flat clusters or single cells
- Clear cytoplasm with well-defined borders and inclusions
- Cytoplasmic glycogen demonstrated by PAS with and without diastase
- No intracellular or extracellular mucin
Mucinous Cystic and Intraductal Papillary-Mucinous (IPM) Neoplasms

1-2% of all pancreatic tumors; 9% of cystic tumors

**WHO**
- Mucinous Cystadenoma
- Mucinous cystic neoplasm with moderate dysplasia
- Cystadenocarcinoma
- IPM Adenoma
- IPMN with moderate dysplasia
- IPM Carcinoma
  - Invasive
  - Non invasive

**Cytological findings**
- Abundant mucin
- Variable cellularity
- Sheets, papillary groups, and single cells
- Absence of atypia, necrosis or mitosis
- Abundant cellularity, discohesion
- Prominent atypia and prominent nucleoli
- Mitosis and necrosis
IPMN

1. Biliary-pancreatic
2. Gastric/null
3. Intestinal
4. Oncocytic (IOPN)

Distinction “Micro IPMN” from “MegaPanIN”

Branch-duct IPMNs
- <3 cm
- No mural nodules
- No irregularities
- No changes
Borderline
The presence of tight epithelial clusters is consistent of at least moderate dysplasia

Carcinoma in situ
Abundant background inflammation and parachromatin clearing correlated with the presence of at least carcinoma in situ

Invasive carcinoma
Necrosis was the only feature found to be strongly suggestive of invasion
Solid Pseudopapillary Tumor

1-2%; low malignant potential; adolescent girls and young women.

Aggressive behavior in some cases with tumor progression

Cytological findings

• Numerous large branching papillary clusters with slender central fibrovascular cores with myxoid stroma

• Monomorphic round to oval nuclei with occasional nuclear grooves and only slight pleomorphism; small nucleoli

• Acinar arrangements with central metachromatic material may impart an adenoid cystic appearance

• Foamy macrophages and necrosis

Pettinato et al Diagn Cytopathol 2002
Canzonieri et al Lancet Oncol 2003
Bardales et al Am J Clin Pathol. 2004
Pelosi et al Diagn Cytopathol 2006
Adamthwaite et al JOP. J Pancreas 2006
Ductal Adenocarcinoma

80-90% of all pancreatic cancers, W-M-P differentiated ADK

Cytological findings

- Marked cellularity
- Monolayer sheets with nuclear overlapping
- Ductal arrangements or isolated tumor cells
- Presence of mitosis
- Inflammatory, stromal cells and foamy macrophages; necrosis
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EUS-FNA Cytology of Exocrine Pancreatic Benign Cystic Tumors

- **Sensitivity 35-90%**
  \[
  \frac{TP}{TP+FN}
  \]

- **Specificity 80-90%**
  \[
  \frac{TN}{TN+FP}
  \]

- **Diagnostic Accuracy 80-90%**
  \[
  \frac{TP+TN}{TP+FP+TN+FN}
  \]

DeWitt Tech Gastrointest Endosc, 2005
Identification of Malignancy by EUS-FNA of Cystic Pancreatic Lesions

- **Sensitivity** 22-95% \(\frac{TP}{TP+FN}\)
- **Specificity** 100% \(\frac{TN}{TN+FP}\)
- **Positive Predictive Value (PPV)** 100% \(\frac{TP}{TP+FP}\)
- **Negative Predictive Value (NPV)** 47-95% \(\frac{TN}{TN+FN}\)
- **Diagnostic Accuracy** 85-90% \(\frac{TP+TN}{TP+FP+TN+FN}\)

DeWitt Tech Gastrointest Endosc, 2005
Conclusions

The diagnostic accuracy of endoscopic ultrasound alone for differentiating between pseudotumoral masses and pancreatic cancer arising from chronic pancreatitis is unsatisfactory. Fine needle aspiration of these tumors significantly improves diagnostic capability.
<table>
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<th>Year</th>
<th>Auth</th>
<th>Cases</th>
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<th>FNA False N</th>
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<td>7 (4%)</td>
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23 year-old male with abdominal pain and weight loss
CT scan: head-body pancreatic mass with extension to the peri-pancreatic tissue
Metastatic disease
ICC-1

CK AE1/AE3

CK7
ICC-2

CHROMOGRANIN-A

SYNAPTOPHYSIN
ICC-3

Ki-67

CEA
ICC-4

**ALPHA-TRYPSIN**

**PAS-D**
Acinar Cell Carcinoma (EUS-FNAC)
Rare tumors: < 2% of all pancreatic tumors; aggressive clinical behavior

Cytological findings

- Cellular smears
- Single cells included stripped naked nuclei
- Loosely cohesive clusters and vague acini
- Monomorphic cells with round to oval nuclei and prominent nucleoli
- Scant to moderate cytoplasm with granularity (best seen on air-dried smears).
- Membrane PAS-D positivity
- ICC:
  - +VE for CKAE1/AE3, CK7, Trypsin, Chymotrypsin, Amilase, Lipase
  - -VE for CEA, CK20, alpha-1-antitrypsin, Ca19.9
  - VARIABLE+/-VE for Chromogranin, Synaptophysin
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1176 Genes

Lipocalin 2 and Tissue-Type Plasminogen Activator

A sufficient amount of high quality RNA can be obtained with EUSFNA

Molecular analysis of EUS-guided FNA samples in pancreatic cancer appears as a valuable strategy for the diagnosis of pancreatic adenocarcinoma.

AIM: To compare gene expression profiles of pancreatic, colorectal adenocarcinoma tissue specimens, human pancreatic and colon adenocarcinoma and leukemia cell lines and normal pancreas samples in order to identify differentially expressed genes and to validate expression of a subset of these genes by RT-PCR and RT-QPCR in endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA) specimens.

RESULTS: RT-QPCR validated expression of LCN2 (lipocalin-2) and for the first time expression of PLAT (tissue-type plasminogen activator or tPA) in malignant pancreatic tissue as compared with normal pancreas and malignant ductal cells of ductal adenocarcinoma.

Conclusions: Molecular analysis of EUS-guided FNA samples in pancreatic cancer appears as a valuable strategy for the diagnosis of pancreatic adenocarcinomas.

World J Gastroenterol 2006 June 7; 12(21): 3344-3351
The ability to obtain cytological specimens by EUS-guided FNA has overcome the difficulty in differentiating between benign vs malignant lesions seen on EUS alone.

Cytological diagnoses may be very accurate (potentially replacing histology). Cytochemistry and immunocytochemistry may be useful adjunctive diagnostic tools as in the reported case of acinar cell carcinoma.
Summing-up 2   PROS

- Sensitivity, specificity, accuracy, NPV and PPV for the diagnosis of pancreatic cancer (>83%, >90%, >85%, >80%, up to 100% respectively in some studies) were optimal for therapeutic planning.

- In staging pancreatic cancer, EUS-guided FNA improves the specificity of lymph node metastasis, with cytological confirmation, as compared to other staging procedures.

Chang, Endoscopy, 2006
One of the most difficult diagnoses is the differentiation between pancreatic carcinoma and chronic pancreatitis, (however FNA PPV of almost 100% and NPV > 85% have been recently reported in a dedicated study*)

Although EUS/EUSFNA can identify pancreatic cancer earlier than any other diagnostic technique, if this could significantly improve survival statistics is still to be determined

Chang, Endoscopy 2006
*Ardengh, JOP, 2007
EUSFNA is going beyond cytology diagnosis to assess for molecular and/or genetic alterations within the tumor tissue, with techniques such as cDNA microarrays which can screen for hundreds of genes simultaneously.

The role of molecular characterization of EUS-FNA samples is in its infancy however the tremendous power of this technology, and promising results in other fields, suggest that this will be an increasing important adjunct to standard cytology.

Pathologists are called on to properly manage these possible diagnostic applications, being familiar with them.
We can afford new technologies by an open-minded approach, in order to widen our professional skill, always maintaining the precious legacy of microscopic diagnosis.

THANK YOU
Dr. R Cannizzaro, Gastroenterology
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Dr. F. De Marchi, Surgical Oncology
Dr. V. De Re, Experimental Oncology
Dr. V Canzonieri, Pathology